# Tropical Microbiology

D. G. Montefiore

K. O. Alausa

O. Tomori

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## D. G. Montefiore

OBE MA MD (Cambridge) FRCPath FMCPath(Nig) FWACP(Path) DipBact(Lond)

Professor of Medical Microbiology, University of Ibadan.
Deputy Provost, College of Medicine, University of Ibadan.
Formerly Dean, Faculty of Basic Medical Sciences & Pharmacy,
College of Medicine, University of Ibadan, Nigeria.

## K. O. Alausa

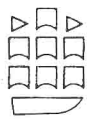
BMedSci MD(Ibadan) DipBact(Manchester) MPH(Harvard) MRCPath FMCPath(Nig) FWACP(Path) CertImmunol(Ibadan) CertEpidem(WHO)

Professor of Epidemiology and Community Health,
University of Ilorin, Nigeria. Formerly Senior Lecturer,
Department of Medical Microbiology,
University of Ibadan, Nigeria.

## O. Tomori

DVM PhD MRCPath

Reader, Department of Virology,
University of Ibadan, Nigeria


CHURCHILL LIVINGSTONE
EDINBURGH LONDON MELBOURNE AND NEW YORK 1984

CHURCHILL LIVINGSTONE
Medical Division of Longman Group Limited

Distributed in the United States of America by
Churchill Livingstone Inc., 1560 Broadway, New York,
N.Y. 10036, and by associated companies, branches and
representatives throughout the world.



First published 1984

ISBN 0 443 02741 2

British Library Cataloguing in Publication Data
Montefiore, D. G.
Tropical microbiology.—(Medicine in the
tropics series)
1. Medical microbiology—Tropics
I. Title II. Alausa, K. O.
III. Tomori, O. IV. Series
616.01'0913 QR46

Library of Congress Cataloging in Publication Data
Montefiore, D. G.
Tropical microbiology.

(Medicine in the tropics series)
Includes index.
1. Medical microbiology—Tropics. I. Alausa, K. O.
II. Tomori, O. III. Title. IV. Series: Medicine in
the tropics. [DNLM: 1. Microbiological technics.
2. Microbiology. 3. Tropical medicine. QW 25 M774t]
QR46.M74 1984 616'.01'0913 83-7715

Printed in Hong Kong by
Sheck Wah Tong Printing Press Ltd.

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# Preface

This book originated from lectures in bacteriology, virology and the immunology of infection given to undergraduate medical and dental students, but it will also be of value to general practitioners and postgraduates working for higher professional qualifications. References to recent publications have been included at the end of each chapter for the benefit of those interested in exploring the subject in greater depth.

Microbiology laboratories are generally scarce in tropical countries, and once away from the main teaching centres most practitioners will not have easy access to these diagnostic services. We have, therefore, emphasised the clinical aspects of the subject, but have included sufficient systematic bacteriology and virology to help understand the pathogenesis of the various infections discussed.

Although most bacterial and viral infections can occur in all parts of the world, climatic and environmental conditions in the tropics result in their being one of the most important causes of disease and death in these areas. An up-to-date knowledge of their causes, prevention and treatment is therefore essential for medical and dental practitioners in tropical countries.

Ibadan, Nigeria, 1984

D. G. M.
K. O. A.
O. T.

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# 1

# Introduction and general properties of bacteria

## TROPICAL IMPLICATIONS OF CLINICAL BACTERIOLOGY

Infections are responsible for much of the illness and death in tropical countries. Most infections are not strictly 'tropical', and those that are—about 5% of the total—comprise mainly malaria and other parasitic diseases. The majority are conditions which can occur in any part of the world, although their prevalence in the tropics may be favoured by poor standards of environmental hygiene, the multitude of potential insect vectors, poor living standards and malnutrition.

Nutrition, concurrent disease and living standards affect the reaction of the host towards infection, and have to be taken into account when deciding on the best means of preventing or treating infectious diseases. Social factors may adversely affect immunisation against infection: for example, in Lagos it was found that few children were effectively immunised and investigations showed that this was largely because mothers could not get to the Infant Welfare clinics and often did not even know where they were. The fact that the tropical climate itself is not entirely responsible for the high incidence of infectious diseases is evidenced by the marked differences in the health of those who are better-off economically and live under relatively good conditions, when compared with the health of the poorer sections of the community.

Like other doctors, the clinical bacteriologist has to take the social environment into account; procedures and advice that may be effective elsewhere can prove ineffective or unrealistic for many people in the tropics.

## THE AGENTS OF BACTERIAL INFECTION

There are many different types of bacteria. Only a few of them are 'pathogenic'—that is, able to cause disease. Clinical bacteriology is concerned with pathogenic bacteria.

Treating or preventing bacterial disease depends on being able to identify the causative agents. This can be done by a variety of methods, which can become quite complex when detailed identification is required. Fully equipped and staffed bacteriology laboratories are scarce in many tropical countries, but much information can be obtained from quite simple tests that do not require any very elaborate supporting services.

## METHODS OF IDENTIFYING BACTERIA

### 1. STAINING REACTIONS

#### a. Gram's staining

Most bacteria can be divided into two main groups by a staining process devised by Gram in 1884.

(i) Prepare a smear of bacteria on a microscope slide and heat fix
(ii) Stain with 1% methyl violet for 1 min
(iii) Mordant with Gram's iodine solution for 30 s
(iv) Wash with water and apply acetone for 1–2 s
(v) Wash with water and counterstain with 1% safranine

Gram-positive bacteria retain the methyl violet, and stain purple.

Gram-negative bacteria are decolourised by the acetone, but are stained by the safranine and appear red under the microscope.

#### b. Ziehl–Neelsen stain

The second most important staining method is used for detecting tubercle bacilli (*Mycobacterium tuberculosis*) and related organisms. These stain poorly by Gram's method because they contain large amounts of lipid in their cell wall, which prevents penetration of the dye. They will, however, take up hot stain in the presence of phenol.

(i) Flood smear with strong carbol-fuchsin (basic fuchsin in phenol)
(ii) Heat until just steaming, for 5 min
(iii) Decolourise with 3% HCl in alcohol
(iv) Counterstain with 1% methylene blue

Tubercle bacilli stain red: all other bacteria and host material stains blue.

### 2. MORPHOLOGY OF BACTERIA

Most bacteria have definite shapes which are maintained by their rigid cell walls. Recognition of these shapes in stained films is an aid

**Table 1.1** Staining reactions and morphology of bacteria

| Staining reaction | Shape | Some bacterial genera |
|---|---|---|
| Gram positive | Round | *Staphylococcus*<br>*Streptococcus* |
| | Rod-shaped | *Clostridium*<br>*Corynebacterium*<br>*Bacillus* |
| | Branching rods | *Actinomycetes* |
| Gram negative | Round | *Neisseria* |
| | Rod-shaped | *Escherichia*<br>*Proteus*<br>*Klebsiella*<br>*Salmonella*<br>*Shigella*<br>*Pseudomonas*<br>*Bacteroides* |
| | Curved rods | *Vibrio* |
| | Spiral rods | *Spirochaetes*<br>*Spirilla* |
| Ziehl–Neelsen positive | Rod-shaped | *Mycobacterium* |
| Do not stain by Gram or Ziehl–Neelsen method | Pleomorphic (variable shape) | *Mycoplasma*<br>*Rickettsia*<br>*Chlamydia* |

Note: * *Mycoplasma* do not possess a cell wall

to identification. The important findings are summarised in Table 1.1.

## 3. CULTIVATION OF BACTERIA

Full identification of bacteria depends on being able to isolate and grow them in pure culture so that their biochemical and other properties can be studied.

Bacteria can be isolated from clinical samples by plating the material on nutrient media which have been solidified by the addition of agar. Many media are available, but these fall into three main classes:

### a. Non-selective media

These are nutrient media which allow the growth of many different types of bacteria without favouring or specifically inhibiting the growth of any particular organism. Examples are: nutrient agar; blood agar; heated blood agar (chocolate agar).

**b. Selective media**

These are nutrient media to which chemicals have been added to inhibit the growth of most bacteria other than the particular pathogen being sought. Examples are: MacConkey agar (contains bile salts which inhibit most bacteria except the *Enterobacteriacae*); deoxycholate citrate agar (inhibits growth of most bacteria except *Salmonella* and *Shigella*).

**c. Indicator media**

These are nutrient media which contain substances—often a carbohydrate substrate and a pH indicator—which detect particular biochemical properties of the bacteria. They are often combined with selective chemicals to produce a selective-indicator medium. Examples are: MacConkey agar (contains lactose and a pH indicator to detect lactose fermentation); mannitol salt agar (contains mannitol, a pH indicator and a high NaCl concentration), and is used to isolate and provisionally identify *Staphylococcus aureus* by mannitol fermentation).

Bacteria plated thinly on solid media will, after incubation, produce single colonies. Each colony results from the growth of a single bacterium, and sub-culture to fresh medium will yield a pure growth which can be used for further biochemical or other tests for identification.

## 4. CONDITIONS OF GROWTH

Some bacteria will only grow if oxygen is excluded from their environment. These are called 'anaerobes', and include the *Clostridia* and the *Bacteroides* as the most important genera. Some, such as *Vibrio* and *Pseudomonas*, will grow only in the presence of oxygen; these are called 'aerobes'. Most other bacteria will grow either with or without oxygen, and are called 'facultative anaerobes'.

## 5. BIOCHEMICAL REACTIONS

In many cases identification requires one to demonstrate the ability of the organism to utilise carbohydrate or other chemical substrate. Carbohydrate utilisation is commonly detected by inoculating the organism into a fluid medium containing the substrate and a pH indicator, and observing for acid formation following incubation.

### 6. SEROLOGICAL REACTIONS

Biochemical tests can sometimes provide a full identification, but it is often necessary to complete this by examining for agglutination or other serological reaction between the bacterial suspension and a specific antiserum which has been prepared by immunising experimental animals with a known strain of the particular micro-organism. If the unknown bacterial suspension reacts with the known antiserum, it means that it must be the same as the organism originally used to immunise the experimental animal.

### 7. OTHER TESTS

Various other tests, such as examination for bacterial motility, or susceptibility to lysis by specific bacteriophage, may be required.

## THE STRUCTURE OF BACTERIA

Bacteria consist essentially of cytoplasm and a circular DNA chromosome surrounded by a cytoplasmic membrane and cell wall, to which various appendages may be attached.

### THE CELL WALL

This is a rigid structure which gives the bacterium its characteristic shape. The detailed structure differs in Gram-positive and Gram-negative bacteria, but in both the basic component is a giant molecule of peptidoglycan. This is a loose meshwork of strands of amino-sugars cross-linked by short peptide chains to give it rigidity and strength. Outside this, there is usually a protein coat or carbohydrate capsule.

### CYTOPLASMIC MEMBRANE

The cytoplasmic membrane lies within the cell wall, kept in close apposition to it by osmotic pressure. It is a semi-permeable structure controlling the ingress and exit of molecules, and is also the site of many cell enzymes concerned with energy production, which are located on membrane invaginations called 'mesosomes'.

### CYTOPLASM

This is enclosed by the cytoplasmic membrane and consists largely of ribosomes, where proteins are synthesised. The circular double-

stranded DNA chromosome is embedded in the cytoplasm, but there is no nuclear membrane as in eucaryotic cells. Smaller circles of double-stranded DNA may also be present as plasmids in the cytoplasmic substance.

## FLAGELLAE

Some bacteria are motile by means of long hair-like processes, the flagellae. These consist of helically wound strands of the protein flagellin, and arise from the cytoplasmic membrane, reaching the exterior by extending through the cell wall.

## FIMBRIAE (PILI)

These are very small hair-like processes present on many Gram-negative bacteria. They arise from the cytoplasmic membrane and extend through the cell wall. Their main function is to attach the bacterium to the surface of host epithelial cells and thus initiate infection. Some Gram-negative bacteria possess a specialised 'sex pilus' which acts as a conjugation tube and allows the passage of plasmid DNA from one cell to another.

## SPORES

Bacteria belonging to the genera *Clostridium* and *Bacillus* form spores under adverse environmental conditions. Spores are very resistant to heat, drying and toxic chemicals, and are a means of survival for long periods. When favourable conditions return, the spore will germinate to form a single vegetative bacterium, which will then proceed to divide and multiply.

## CAPSULES

Some bacteria form capsules as a mucoid layer surrounding the cell wall. The ability to form a capsule is genetically determined and easily lost on sub-culture in the laboratory. Capsules protect the bacterium from ingestion by host phagocytic cells, and are therefore an important factor in the virulence of an infecting bacterium.

The basic cell structures are illustrated in Figure 1.1

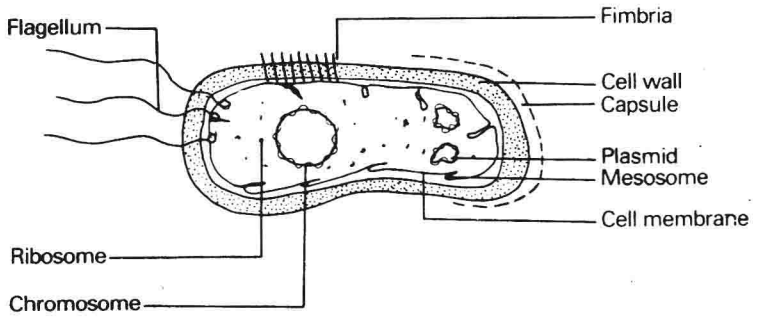


**Fig. 1.1** The bacterial cell

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# 2

# Collection of samples for examination

## TROPICAL IMPLICATIONS FOR SAMPLE COLLECTION

A sample of the appropriate body fluid, material or exudate must first be collected and transported to the laboratory before any diagnostic tests can be undertaken. In theory this procedure should be the same world-wide. In practice, unforeseen difficulties may arise in the tropics.

### 1. THE REQUEST FORM

All samples must be accompanied by a request form giving details of the patient, his location, his provisional diagnosis and previous treatment. In theory this is simple; in practice, patients in the tropics have usually tried various treatments before attending hospital, and are likely to have taken at least some doses of antibiotics obtained from the chemist shop or elsewhere. They will usually deny this, but as the results of bacteriological culture can be seriously affected by prior antibiotic treatment, the information should be sought and included if possible.

There may also be difficulty with names and patients may sometimes give different names on different occasions. A hospital or clinic number, recorded on a card which the patient presents on repeat attendances, should always be included on the request form.

### 2. THE SPECIMEN CONTAINER

The specimen container must be labelled with the name of the patient, his location and hospital number, to match the details given on the request form.

Most samples must be taken into sterile containers, but outpatients may bring urine samples in an extraordinary variety of bottles, all of which will be unsterile. Some may contain traces of disinfectant, while others may have been washed out with dirty

water. Most of these samples will have to be rejected and a new sample taken into a suitable bottle.

### 3. DETERIORATION OF SAMPLES

Postal services in the tropics are usually slow and the question of receiving samples by this route seldom arises. But even within a hospital, the high environmental temperature of the tropics can lead to sample deterioration. This particularly affects pus and exudates taken on cotton wool-tipped swabs, which dry rapidly so that only the most resistant organisms survive by the time the specimen reaches the laboratory.

Urine samples may also be affected. Urine is an excellent culture medium and small numbers of contaminating organisms can multiply rapidly at tropical temperatures, making the results of examination impossible to interpret. Refrigeration of samples pending delivery to the laboratory is seldom possible, but a simple alternative is to use urine containers with boric acid as a preservative. Approximately 0.5 g of boric acid per 25 ml of urine will prevent bacterial multiplication, but will not kill the organisms. It will also preserve cells and other formed elements for several hours at room temperature.

## GENERAL SAMPLING METHODS

### 1. FAECES

Sterile bottles are not required for collection of faecal samples. Plastic or waxed-cardboard containers are cheap and usually available. The major consideration is that they should have a well fitting screw type lid to prevent leakage of fluid or semi-solid stool.

### 2. URINE

A sterile container is essential. The most suitable is a Universal bottle containing 0.5 g of boric acid.

### 3. TISSUE EXUDATES

Where possible, pus should be collected in a sterile Universal container. Usually cotton-tipped swabs are used when there is no pus. The swab should be replaced in its outer tube and sent to the laboratory as soon as possible to prevent loss of delicate bacteria due